

Frequency of *FCGR3B* Alleles in Thai Blood Donors

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Background: Human neutrophil antigens (HNAs) are involved in autoimmune and alloimmune neutropenia and transfusion-related acute lung injury. The HNA-1 system is important in immunogenetics, and allele frequencies have been described in different populations. This study investigated the frequency of *FCGR3B* alleles encoding HNA-1a, HNA-1b, and HNA-1c among Thai blood donors and compared these frequencies with those previously reported for other populations.

Methods: Eight hundred DNA samples obtained from unrelated healthy blood donors at the National Blood Centre, Thai Red Cross Society, Bangkok, and the Blood Bank, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, were included. Samples were simultaneously typed for each *FCGR3B* allele using an in-house polymerase chain reaction with sequence-specific primer (PCR-SSP) technique.

Results: The frequencies of *FCGR3B**1, *FCGR3B**2, and *FCGR3B**3 alleles in central Thai blood donors were 0.548, 0.452, and 0.004, respectively; only *FCGR3B**1 and *FCGR3B**2 alleles were found in northern Thai blood donors (0.68 and 0.32, respectively). Compared with other Asian populations, central Thais had higher frequencies of the *FCGR3B**2 allele ($P < 0.001$), while the frequencies of the *FCGR3B**1 and *FCGR3B**2 alleles in northern Thais were similar to those previously reported in Taiwanese and Japanese populations. In contrast, the frequencies of the *FCGR3B**1 and *FCGR3B**2 alleles in the northern Thai population were statistically different from those observed in central Thai, Korean, German, and Turkish populations.

Conclusions: *FCGR3B* allele frequencies were significantly different between central and northern Thai blood donors. Our in-house PCR-SSP method is a simple, cost-effective, and convenient method for *FCGR3B* allele detection.

Key Words: *FCGR3B*, Allele frequencies, Thais

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INTRODUCTION

In Thailand, the demographic Thai populations include the central, northeastern, northern, and southern Thais. The northern Thai population is one of the major subgroups of ethnic Thais and has close cultural and historic connections with the populations of southern Chinese, Myanmar and Laos. A previous study on the HLA system in 5 ethnic Thai populations recruited from central, northeastern, and northern Thailand demonstrated that

the *HLA-DRB1**15:02 allele appeared to be dominant in Thais; however, the *HLA-DRB1**15:01 and the *HLA-DRB1**09:01 alleles were relatively more common in northern Thais than other ethnic Thais [1]. This evidence supports the hypothesis that the heterogeneous southern populations of East Asia are genetically derived from the population of Southeast Asia [2, 3].

Human neutrophil antigens (HNA) are polymorphic structures located in the membranes of neutrophils [4]. Currently, 5 HNA systems (HNA-1 to HNA-5), have been characterized [5, 6]. In

the HNA-1 system, 3 antigens, HNA-1a, HNA-1b and HNA-1c, are located on the neutrophil low-affinity Fc- γ -IIIb receptor (CD 16) glycoprotein [7]. Because Fc- γ -IIIb is the most immunogenic glycoprotein on the neutrophil membrane, antibodies to these antigens are frequently implicated in alloimmune and autoimmune neutropenia and transfusion-related acute lung injury (TRALI) [8-12].

The Fc- γ -IIIb receptor is encoded by the *FCGR3B* gene and occurs in 3 polymorphic forms representing the human neutrophil antigens, that is, HNA-1a, HNA-1b, and HNA-1c, which are encoded by the *FCGR3B*1*, *FCGR3B*2*, and *FCGR3B*3* alleles, respectively [13]. In addition to their clinical significance, the *FCGR3B* allele frequencies could be used as an additional marker in population studies [14-16]. Moreover, the HNA genetic system of Thai populations has not yet been adequately studied. Therefore, this study aimed to determine the *FCGR3B*1*, *FCGR3B*2*, and *FCGR3B*3* allele frequencies encoding HNA-1a, HNA-1b, and HNA-1c, respectively, in Thai blood donors by using a PCR sequence-specific primer (PCR-SSP) technique to compare the allele frequencies with those previously reported in other populations.

METHODS

1. Study population

Peripheral venous blood was collected in EDTA-anticoagulated vacutainer from 800 unrelated healthy Thai blood donors. Five hundred samples, that is, 300 samples from our previous study [17] and an additional 200 samples, were from the National Blood Centre, Thai Red Cross Society, Bangkok. In addition, 300 samples were from the Blood Bank, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Written informed consent was obtained from each subject. This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand. Genomic DNA was extracted from peripheral blood samples using the Genomic DNA extraction kit (REAL Genomics, RBCBio-science, Taipei, Taiwan) and by a salting out method, as previously described [18], and was then stored at -20°C until use for genotyping.

2. *FCGR3B* DNA amplification using PCR-SSP

Known HNA-1a, HNA-1b, and HNA-1c DNA samples were provided by Dr. Núria Nogués, Laboratori d'Immunohematologia, Banc de Sang i Teixits, Passeig Taulat, Barcelona, Spain. A PCR-SSP technique was performed as previously described [9]

Table 1. Primers used for *FCGR3B*-specific DNA amplification [4, 9]

Primers	Sequence (5'→3')	Product size (bp)
HNA-1a (F)	CAGTGGTTTCACAAATGTGAA	141
HNA-1a (R)	ATGGACTTCTAGCTGCAC	
HNA-1b (F)	CAATGGTACAGCGTGCTT	219
HNA-1b (R)	ATGGACTTCTAGCTGCAC	
HNA-1c (F)	AAGATCTCCCAAAGGCTGTG	191
HNA-1c (R)	ACTGTCGTGACTGTGTCAT	
HGH (F)	TGCCTTCCCAACCATTCCCTTA	434
HGH (R)	CCACTCACGGATTCTGTGTGTTTC	

Abbreviations: HNA, human neutrophil antigens; HGH, human growth hormone; F, forward; R, reverse.

with some modifications. The sequences of the primers used for HNA genotyping are shown in Table 1. The specific primers were similar to those previously described [4, 9]. Briefly, 2 μ L of genomic DNA (50 ng/ μ L) was amplified in a total volume of 10 μ L with 0.5 μ M of HNA-1a forward (F) and reverse (R) primers for *FCGR3B*1* genotyping, 0.5 μ M of HNA-1b (F) and (R) primers for *FCGR3B*2* genotyping, and 0.5 μ M of HNA-1c (F) and (R) primers for *FCGR3B*3* genotyping. The human growth hormone (HGH) gene was coamplified using 0.125 μ M HGH (F) primer and 0.125 μ M HGH (R) primer and was used as an internal control. PCR was performed with 5 μ L of DreamTaq DNA polymerase (Thermo Fisher Scientific Inc., Glen Burnie, MD, USA) consisting of 2X DreamTaq Green buffer, 0.4 mM of each of the dNTPs, and 0.4 mM MgCl₂ in a G-STORM GS1 thermal cycler (Gene Technologies Ltd., Somerton, UK). The cycling parameters for the PCR program were as follows: 1 cycle of 300 sec at 95°C; 30 cycles each of 30 sec at 95°C, 60 sec at 57°C, and 30 sec at 72°C; and final extension for 5 min at 72°C. The product was then kept at 4°C until use for analysis. After amplification, the PCR products were analyzed on a 2.0% agarose gel using 1X Tris borate ethylenediaminetetraacetate (TBE) buffer containing SYBR Green I nucleic acid gel stain (Invitrogen, Grand Island, NY, USA) and were visualized under ultraviolet (UV) illumination (Fig. 1).

3. Statistical analysis

Gene frequencies were calculated by gene counting as previously described [4, 19]. The Chi-square test was used to evaluate whether the observed genotype frequencies were in agreement with the expected ones under Hardy-Weinberg equilibrium. Genotype and allele frequencies of *FCGR3B*1*, *FCGR3B*2*, and *FCGR3B*3* were compared between different populations by using the Chi-square test. *P* values of less than 0.05 were consid-

ered statistically significant.

RESULTS

The *FCGR3B* genotypes and allele frequencies among different populations are shown in Table 2. For the central Thai blood donors, the *FCGR3B**1 allele was most frequently detected (0.548 [548/1,000]), followed by *FCGR3B**2 (0.452 [452/1,000]), with *FCGR3B**3 being the least frequent (0.004 [4/1,000]). *FCGR3B**1/*FCGR3B**2 was the most frequent genotype (321/500), followed by *FCGR3B**1/*FCGR3B**1 (112/500) and *FCGR3B**2/*FCGR3B**2 (63/500). On the other hand, only 2 alleles, *FCGR3B**1 and *FCGR3B**2, were found in the northern Thai population, with allele frequencies of 0.677 (406/600) and 0.323 (194/600), re-

spectively. In this population, *FCGR3B**1/*FCGR3B**1 was the most frequent genotype (140/300), followed by *FCGR3B**1/*FCGR3B**2 (126/300) and *FCGR3B**2/*FCGR3B**2 (34/300). We did not find any HNA-1 null subjects among the 800 samples investigated in the present study. Moreover, the *FCGR3B* locus was in Hardy-Weinberg equilibrium in Thai populations. The genotype frequency distribution of the central Thai population was also compared with that of the northern Thai population and other studies previously reported [9, 14, 16, 20, 21]. The *FCGR3B**1 and *FCGR3B**2 allele frequencies of the northern Thais did not differ from those of the Taiwanese and Japanese populations [9, 14]. Interestingly, the *FCGR3B**1 and *FCGR3B**2 allele frequencies were significantly different from those observed in the central Thai, German, Turkish, Tunisian, and Korean populations ($P < 0.01$) [16, 20, 21].

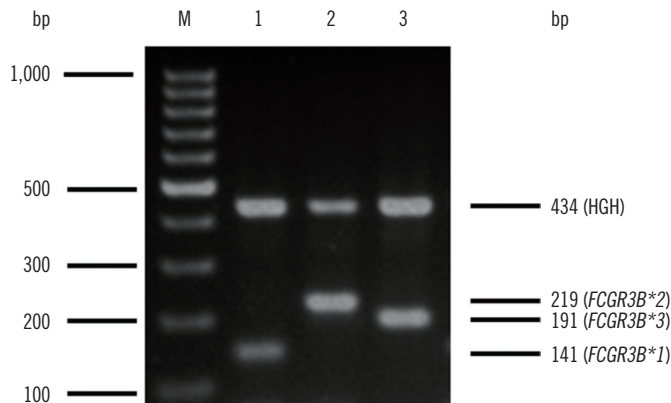


Fig. 1. PCR sequence-specific primer analysis of the *FCGR3B**1-, *FCGR3B**2-, and *FCGR3B**3-positive individuals. The 141-bp, 219-bp, and 191-bp bands represent the specific PCR products and the 434-bp HGHI bands are the internal controls. PCR products were analyzed on a 2.0% agarose gel stained with ethidium bromide. The DNA size standard (100-bp ladder marker; Fermentas, Carlsbad, CA, USA) is shown in lane M.

DISCUSSION

In this study, the *FCGR3B* alleles encoding the HNA-1a, -1b, and -1c antigens were analyzed by the in-house PCR-SSP technique, because the commercial kit for HNA-1a, HNA-1b, and HNA-1c genotyping is expensive and is not available in Thailand. Although the PCR-SSP technique is not suitable for high-throughput genotyping, it is widely used in routine laboratory examinations for both HLA and HNA genotyping because of its simplicity. Moreover, in order to simultaneously read the HNA-1 genotyping results, the same PCR conditions were used for each *FCGR3B* allele detection, which makes this technique less time consuming than the PCR-SSP technique previously reported [9]. Additionally, the cost of this in-house PCR-SSP technique is approximately only 1 US dollar per test.

It was found that the *FCGR3B**1 allele was the most common

Table 2. *FCGR3B* genotype and allele frequencies among different populations

Population	Genotype frequencies (N)					Allele frequencies		
	*1/*1	*1/*2	*2/*2	*2/*3	*1/*2/*3	*1	*2	*3
Northern Thais (N=300)	0.467 (140)	0.420 (126)	0.113 (34)	0.00 (0)	0.00 (0)	0.677	0.323	0.000
Central Thais (N=500)	0.224 (112)	0.642 (321)	0.126 (63)	0.002 (1)	0.006 (3)	0.548 [‡]	0.452 [‡]	0.004
Korean [20] (N=200)	0.285 (57)	0.590 (118)	0.125 (25)	0.00 (0)	0.00 (0)	0.580 [†]	0.420 [†]	NA
Taiwanese [9] (N=138)	0.457 (63)	0.449 (62)	0.094 (13)	0.00 (0)	0.00 (0)	0.681	0.319	0.000
Japanese [14] (N=400)	0.365 (146)	0.515 (206)	0.120 (48)	0.00 (0)	0.00 (0)	0.623	0.377	0.000
German [21] (N=119)	0.151 (18)	0.479 (57)	0.361 (43)	0.00 (0)	0.009 (1)	0.395 [‡]	0.605 [‡]	0.025
Turkish [21] (N=118)	0.161 (19)	0.517 (61)	0.305 (36)	0.00 (0)	0.017 (2)	0.428 [‡]	0.572 [‡]	0.030
Tunisians [16] (N=62)	0.145 (9)	0.403 (25)	0.291 (18)	0.00 (0)	0.160 (10)	0.347 [‡]	0.573 [‡]	0.080

[†] $P < 0.01$; [‡] $P < 0.001$.

Abbreviations: *1, *FCGR3B**1; *2, *FCGR3B**2; *3, *FCGR3B**3; NA, not assessed.

allele in 800 Thai blood donors. This finding confirms that the *FCGR3B*1* allele is more frequent than the *FCGR3B*2* allele in Asian populations, Afro-Brazilians, and Native Americans [4, 6, 7, 9, 13, 14]. On the other hand, the *FCGR3B*1* and *FCGR3B*2* allele frequencies in the northern Thai population were significantly different from those in the central Thai and Korean populations [20]. The *FCGR3B*3* allele, which is considered to be a rare allele in Asian populations [9], was found only in central Thai blood donors, similar to the findings of a previous study [17]. This allele was present in 9.09% of Tunisian blood donors and only in 5% of European populations [16].

HNA-1a individuals with the *FCGR3B*1/FCGR3B*1* genotype are reported to be more susceptible to idiopathic pulmonary fibrosis, whereas HNA-1b individuals with the *FCGR3B*2/FCGR3B*2* genotype have a higher susceptibility to periodontal diseases and chronic periodontitis because of differing reactivity to opsonised bacteria [22]. Therefore, diseases associated with HNA-1 antigens found in the northern Thai population are different from those for the central Thai population. Further studies are required on the association of HNA-1 and disease susceptibility. Additionally, a higher frequency of the *FCGR3B*1* allele and a lower frequency of the *FCGR3B*2* allele were found in northern Thai blood donors compared with the central Thai population, suggesting that northern Thais would be more susceptible to HNA-1a alloimmunization than the central Thais, similar to other Asian populations [14, 20].

Our results demonstrate that the *FCGR3B*1* and *FCGR3B*2* allele frequencies significantly differ between the central and northern Thai populations. However, the allele frequencies in the northern Thai population are closely related to those for the Taiwanese and Japanese populations. In addition, further studies on HNA-3, HNA-4, and HNA-5 gene frequencies would be beneficial in order to develop a database of HNA characteristics in Thai populations.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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